

ABSTRACTS

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Transplantation Antigens in Subcellular Fractions

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Recent results from this laboratory have established that microsomal fractions of lymphoid cells are potent transplantation antigens. The current study was undertaken to determine: 1) the antigenic activity of the mitochondrial fraction and cell sap of liver, kidney, and spleen cells; and 2) the effect of RNAase and ultrasound wave treatment of spleen microsomes. Subcellular fractions were prepared by differential centrifugation of tissue homogenates in 0.25 M sucrose. Mice of strain A/J served as donors of the antigens and test grafts while strain CBA served as recipients. The antigenic potency of various fractions was assessed by their ability to sensitize recipient mice as measured by accelerated rejection of subsequent test skin grafts. The results

showed the following: 1) Mitochondrial fractions of spleen cells are as potent as microsomal fractions in the induction of homograft immunity. Mitochondrial material derived from 25 mg. spleen tissue represents the lower limit of antigenic activity. Liver and kidney mitochondrial fractions were devoid of antigenic activity as were kidney microsomes. No detectable transplantation antigens were present in the cell sap under the conditions of these experiments. 2) Spleen microsomes retained their antigenic activity following exposure to RNAase or brief treatment with ultrasound waves.

In conclusion, cytoplasmic transplantation antigens appear to be associated with the particulate components of the cytoplasm, i.e., microsomes and mitochondria.

Blood Coagulation and Fibrinolytic Enzyme Studies During Cyclical and Continuous Administration of Progestational Agents

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Contradictory statements have been reported on the changes in blood coagulation and the fibrinolytic enzyme system in patients receiving oral contraceptives. Ten volunteers were studied for three consecutive cycles, using a double-blind technique. The following medications were given, each for 1 month: 1) medroxyprogesterone acetate and ethinyl estradiol, 2) norethynodrel and mestranol, and 3) placebo. The blood coagulation and fibrinolytic enzyme system were assayed on days 1, 3, 5, 8, 12, 15, 20, 24, 26, and 28 of each cycle. Group comparisons were made and each patient served as her own control. Over a period of 1 month's use, there were no differences among any of the regimens. However, patients receiving a continuous high dose of Enovid (15 to 60 mg. daily), for a period of several

months, had significant increases in plasminogen concentration, fibrinogen level, and factor VII activity.

In a case of cyclic thrombocytopenia receiving progestational steroids, the cyclic pattern remained unchanged, though the platelet count moved to a higher level within the normal range.

The conclusion reached was that sex steroids in pregnancy are responsible for the increase in blood coagulation factors. A dose-time relationship is evident. Low doses for a short time result in no change, whereas high doses produce an increase in coagulation factors.

It remains to be determined whether low doses given cyclically over a long period of time also result in an increase in blood coagulation factors.

Necrosis in the Fetus and Congenital Malformations

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Necrosis, inflammation, and repair may affect normally differentiated tissues and organs in the fetus. Resulting malformations are often misinterpreted as produced by faulty embryogenesis. Morphologic observations of human jejuno-ileal atresia and review of pathogenic theories suggest that necrosis is the basic common lesion. Early inflammation was studied in the skin of rat fetuses burned in utero at 15 to 21 days of gestation. The ileum was ligated or devascularized at 20 to 27 days of gestation in 50 rabbit fetuses and the results observed 2 to 8 days later. The outcome is affected by two main factors: the *extent* of insult determines the chances of repair without or with minimal tissue loss, the *timing* determines the type of tissue response. The pattern of inflammation changes with fetal age: rapid mesenchymal and epithelial repair, absent or slight topical vasodilatation and lack of migratory cells in young fetuses; vascular

response as mast cells appear, leukocytic infiltration, and slower fibrous and epithelial repair in older fetuses. Most types of atresia observed in man were reproduced, ranging from focal lesions after minimal vascular damage to segmental defect of gut and mesentery after extensive devascularization. Retardation of growth in ischemic tissues is apparently another factor and may play a role in the pathogenesis of "hypoplasia" or stenosis of other hollow structures. Many factors may produce focal lesions with secondary malformation in human fetuses. Those recognized in our material are infectious and vascular. Infarcts were found in mechanical lesions (decubitus, volvulus, intussusception, herniation), fetal thromboembolism, fetal anemia, and vascular anomalies. (*This investigation was supported by Public Health Research Grant HED 00743 from the National Institutes of Health, Bethesda, Md.*)

Guanidinosuccinic Aciduria: An Alternate Route of Ammonia Detoxification

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In 1963, we reported the isolation of a substance from urine of uremic subjects subsequently identified as guanidinosuccinic acid. The present report amplifies these observations and introduces data concerning its metabolic source and its role in the uremic syndrome. Isolation of the material, along with arginine and glucosamine, was performed on urine from uremic patients and compared with that from control subjects. Quantitation and identification were accomplished by means of both column chromatography and paper electrophoresis. Plasma separations of these substances were carried out by differential precipitation. Rats were rendered uremic by subtotal nephrectomy and urine fractionation ob-

tained by methods similar to those in humans. Results show that the substance can be recovered exclusively from the urine of uremic subjects. It is isolated in substantial amounts irrespective of the blood ammonia and unaltered by gastrointestinal sterilization. Electrophoresis of plasma filtrates failed to show the presence of guanidinosuccinate in instances where it was patently demonstrable in the urine implying that the material is not normally resorbed by the renal tubule. Preliminary observations on animal urine using isotopically labeled precursors suggests that this substance represents an alternate to the Krebs-Hensleit cycle for the removal of nitrogen whenever plasma urea levels become excessive.

Further Studies of an Insulinlike Thymic Factor

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Previous histologic studies of beef and pork thymus have described an insulinlike material localized to the reticular cells. Acid-ethanol extracts of these tissues demonstrated immunoassayable insulin activity of approximately 4 mU./g. by the Morgan and Lazarow technique. The present investigation was designed to extend these previous observations. Accordingly, double diffusion in Ouchterlony plates was performed, utilizing extracts of beef and pork pancreas, thymus, liver, lung, and spleen and an homologous anti-insulin antibody. In addition, liver homogenates containing insulinase activity were incubated with extracts of pancreas and thymus at 37° C., heated to 62° C. to destroy the enzyme; frozen; and

then, along with suitable controls, subjected to immunoassay. The results of these studies can be summarized as follows: 1) insulin antibody produces a line of precipitation with thymic and pancreatic extracts but not with lung, liver or spleen; 2) lines of precipitation between pancreas, thymus, and insulin antiserum reveal reactions of either partial or nonidentity; and 3) insulinase is capable of degrading the immunoassayable activity of thymus and pancreas. These findings offer further support of previous studies that demonstrated an insulinlike material in the thymus. The relationship between this substance and the lymphocyte-stimulating factor remains to be elucidated.

Effect of Simple Sugars on the Morphology and Growth Patterns of Mammalian Cell Cultures

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Recent work suggests that cell-to-cell associations are mediated through the surface sugars of one cell with complementary sites on another. When mammalian cells in tissue culture are grown in the presence of simple sugars found in the heterosaccharides of mammalian cell surfaces, there is a selective effect of individual sugars on the cell morphology and pattern of growth of certain cell lines. For example, the attachment of 3T3 mouse fibroblasts to each other in tissue culture is prevented when L-fucose is added to the medium. This effect was not observed when other sugars that occur on cell surfaces were added to the medium nor when

D-fucose was used. The findings suggested that L-fucose caused this alteration in the pattern of growth by substituting for cell contact since the cells altered by sugars underwent the same morphological and metabolic changes which occur during contact inhibition. When the same cell line, 3T3, was infected with either SV₄₀ virus or polyoma virus, the cells lost their usual high degree of contact inhibition and assumed many of the growth characteristics of neoplastic cells. Correspondingly, the effects of L-fucose on 3T3 cells altered by oncogenic viruses were greatly reduced.

Characterization of HeLa Cell Ribonucleic Acid

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The study of synthesis and function of various classes of RNA in cultured animal cells has been under way in this laboratory for several years. Among the findings that will be described are: 1) Ribosomal RNA is transcribed as a large molecule that is subsequently cleaved or dissociated into the

correct sizes. The appearance in the cytoplasm of ribosomes containing newly formed ribosomal RNA will be discussed. 2) Messenger RNA which functions in the cytoplasm has been identified and its movement into the cytoplasm investigated.

The Oxyhemoglobin-Hemoglobin Transition in Aqueous Solution

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Physical chemical studies have been performed on aqueous solutions of human hemoglobin A, with reference to the molecular changes associated with deoxygenation. Data from light-scattering and sedimentation-velocity experiments indicate that deoxygenation of oxyhemoglobin results in an aggregation involving a small fraction of the total protein. The nature of the phenomenon was further studied using density gradient ultracentrifugation. In 2 *M* CsCl, oxyhemoglobin is distributed as a soluble fraction having a buoyant density of 1.25 g./ml. and an insoluble aggregate, the extent of which is concentration and temperature dependent. The soluble fraction has a gaussian distribution indicative of monodispersity in density population, with a weight-average molecular weight of 40,000 to 50,000. These and other data indicate a partial dissociation of oxyhemoglobin into equivalent subunits in 2 *M*

CsCl. Deoxygenation of the protein solution, with incomplete conversion to reduced hemoglobin, results in the appearance of an additional component having a distribution that overlaps that of oxyhemoglobin and has a buoyant density of 1.23 g./ml. Associated with the low-density component is another aggregate, probably a low *n*-mer, that undergoes a volume contraction and is absorbed into the high-density polymer band following increase in temperature from 6° to 25° C. The occurrence of the low-density component indicates that reduction of oxyhemoglobin in aqueous solution results in a volume expansion of the macromolecule. The change seems to be a fundamental property of the hemoglobin molecule. The significance of the volume expansion and of the aggregation phenomena in relation to the sickling process in erythrocytes is evident and is under study.

Synthesis and Breakdown of Lecithin by Phagocytic Cells

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De novo synthesis of phospholipid by the Kennedy-Weiss pathway is stimulated slightly during phagocytosis but may be insufficient to account for the marked increase in formation (and breakdown) of membranes seen morphologically. In this study, alternate pathways of biosynthesis of lecithin (PC) have been compared in homogenates of two phagocytic cells that differ in their intermediary metabolism: rabbit polymorphonuclear leukocytes (WBC) and alveolar macrophages (MAC). Direct conversion of lysolecithin (LPC) to PC has recently been shown to occur in various tissues in 2 ways: 1) $\text{LPC} + \text{fatty acid} \rightarrow \text{PC}$, in the presence of ATP and CoA; and 2) $2 \text{ LPC} \rightarrow \text{PC} + \text{glycerophosphorylcholine (GPC)}$, a transfer reaction in which one LPC donates its fatty acid to another LPC. The occurrence of these pathways was established employing biosynthetically labeled LPC carrying ^{32}P in the P-choline moiety and ^{14}C in the fatty acid. The ratio of ^{14}C to ^{32}P was determined in the PC formed and in the remaining LPC, allowing the contribution of each pathway to be assessed. Both WBC and MAC synthesize PC by direct acylation of LPC (reaction 1). This reaction can be

demonstrated at low LPC concentration, at physiologic pH. At higher LPC concentrations and at acid pH, WBC also manifest reaction 2. In MAC homogenates this reaction accounts for little PC formation at physiologic pH and even less at acid pH. Centrifugation at 100,000 g for 1 hour removes most acylating activity from the supernatant fraction of both homogenates; whereas reaction 2 remains fully active in the supernatant fraction of the WBC homogenate. In addition, both WBC and MAC contain phospholipase A and lysolecithinase activities with acid and approximately physiologic pH optima respectively. LPC is a powerful membrane-lytic agent. Thus these phagocytic cells can produce LPC as well as remove it. Removal can occur in two ways: by hydrolysis to GPC and by resynthesis of PC. These pathways of formation and breakdown of PC may have a role in the increased turnover of membrane observed during phagocytosis. (*This investigation was supported by Public Service Research Grant AM 15472 from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.*)

Rabbit Leukocyte Cultures: Effect of Phytohemagglutinin and Growth of Macrophages

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Phytohemagglutinin (PHA) *in vitro* cultures causes transformation of human lymphocytes into blastlike cells capable of RNA, DNA, γ -globulin synthesis and division. The present study was undertaken to examine the *in vitro* effect of PHA on rabbit lymphocytes. Growth of macrophages (M) in these cultures provided an oppor-

tunity to investigate their origins. Cultures of rabbit peripheral blood (PB) and thoracic duct lymphocytes (TDL) were prepared and harvested after 72 to 120 hours according to Bach and Hirschhorn's technique. In experiments devised to determine the origins of M, PB was relatively cleared of monocytes by filtering it through glass

PB	Mitosis (%)	Lymph (%)	Blast (%)	M (%)	Poly (%)
Control	0.07	74	2.3	12.2	11.5
PHA	1.4	41.4	41.5	11.0	6.1
TDL					
Control	0.14	96.7	3.3		
PHA	1.32	72	28		

wool. There was an appreciable difference as to the number of blastlike cells and mitotic figures among 72-hour control and PHA-stimulated cultures. In both of them large cells with bilobed nuclei containing big granules and vacuoles were observed. They were determined to be M by the fact that they phagocytosed opsonized bacteria. TDL cultures showed similar response to PHA. No growth of M were ever observed in them. The accompanying table summa-

rizes the numerical relationship between the average figures for different cell types. In contrast to 72-hour cultures, 5-day-old cultures showed spontaneous transformation and division of lymphocytes without PHA. When leukocytes filtered through glass wool were cultured, a decrease in the number of M relative to the decrease in the number of monos were observed. This, along with the findings from TDL cultures, pointed to a monocyte-macrophage relationship.

The Abscopal Effect of X Irradiation of the Body (Testes Shielded) on Semen Production in the Guinea Pig

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In view of the significance of our report of an abscopal effect of irradiation of the body (testes shielded), which depressed sperm output in the guinea pig (*Radiat. Res.* 24:67-80, 1965), this work was repeated, using a larger group of animals. Semen was collected from 72 animals for 17 weeks before irradiation and 3 treatments were applied—control (0 r); head and body irradiated with 300 r (testes shielded); and testes irradiated with 15 r (head and body shielded). The 15 r to the testes was applied because we had demonstrated that when 300 r was applied to the body (testes shielded), \pm 15 r was scattered to the testes. Semen was collected for 14 weeks postirradiation, and the presence of an abscopal effect, which depressed sperm output, was demonstrated and confirmed. Inasmuch as it has been suggested that the abscopal effect of irradiation on other organs, e.g., the thymus,

is mediated through a depression in food intake after irradiation, we studied the effect of 300 r of x irradiation on body weight and food and water consumption in 24 animals. Food consumption (g. food/100 g. body weight/24 hours), water consumption (g. water/100 g. body weight/24 hours), and body weight were measured daily for 45 days before and for 57 days after 300 r of whole-body x irradiation. There was no effect of this dose of radiation on food or water consumption or on body weight. The presence of an abscopal effect of irradiation of the body (testes shielded) has been confirmed, and it has been demonstrated that this effect on spermatogenesis is not mediated through changes in food or water intake. (*This investigation was supported by Public Health Research Grant GM-06880-06 from the National Institute of General Medical Sciences, Bethesda, Md.*)

*Persistence of Individual Specific Transplantation Antigens
in Long-Term Cultures of Rabbit Skin*

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Two cell lines RS7 and RS10 obtained from hybrid rabbit skin biopsies have been grown *in vitro* for 13 months while the donor rabbits have remained alive. Both lines have remained diploid with a normal karyotype.

A suspension of 50×10^6 skin cells grown *in vitro* in 3 ml. of serumless growth medium was administered as 60 intradermal injections spaced over the body of recipient rabbits. Each skin cell recipient received "specific" full thickness skin allografts from the donor of the cell line and from a "non-specific" donor 8 days after the sensitizing skin cell inoculations. Daily observations of

specific and nonspecific skin allografts were recorded for experimental and control animals.

The mean rejection time for "specific" full thickness skin allografts on sensitized donors was significantly shorter than that of control first set grafts (6.3 versus 9.7 days). A shortened mean survival (7.3 days) of "nonspecific" challenge grafts was also noted.

The data suggest that mammalian cells grown *in vitro* offer a constant source of uniform antigen supply from a live hybrid animal with a unique quota of transplantation antigens.

Analysis of Carotid Circulation

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The carotid circulation may be studied by analysis of the ocular pulse. When a continuous recording of intraocular pressure is obtained with a cannula in the anterior chamber or a tonometer on the intact eye, a pulsatile curve is obtained. Acute and chronic common and internal carotid occlusion alter the nature of this curve in a predictable manner. Acute compression of the homolateral common carotid reduces the amplitude of the homolateral ocular pulse and minimally affects the contralateral

pulse. The homolateral pulse is reduced in chronic closure and is profoundly altered by compression of the contralateral carotid. In carotid cavernous sinus fistulae, the appearance of the tonogram is diagnostic of the condition, and the efficacy of the therapy may be followed by ocular pulse studies. Analysis of the ocular pulse aids in the diagnosis of carotid occlusion, insufficiency, and fistula, and provides information second only to angiography.

Sulfhydryl Group Reactivity and Rheumatoid Arthritis

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Observations have been made concerning biochemical abnormalities in the blood and urine of patients with rheumatoid arthritis and on the chemical reactivity of drugs used either in the specific treatment of rheumatoid arthritis (i.e., gold and chloroquine) or drugs that may induce arthritis (i.e., hydralazine). These observations may be summarized as follows: 1) Gold and chloroquine were found, unlike 118 other randomly selected drugs, to be potent suppressors of ionized SH group reactivity. 2) Hydralazine and 3-OH-anthranilic acid unlike 231 other common drugs or biochemicals were found in the presence of copper to be potent splitters of the disulfide (SS) bond. It is known that 3-OH-anthranilic acid is present in increased amounts in the urine of patients with rheumatoid arthritis. 3) Approximately 50% of 80 patients with rheumatoid arthritis excreted in timed urine collections amounts of copper ligands in excess of all but 5%

of 215 patients with unrelated diseases ($p < 0.001$). Increased copper ligand excretion was not associated with inflammation unrelated to rheumatoid arthritis or with the administration of aspirin. The major constituents in urine with this copper ligand reactivity have been identified as histidine and 3-methyl-histidine. Both of these compounds are unusually potent inhibitors of the splitting of the SS bond by 3-OH-anthranilic acid. Serum histidine concentrations have been reported to be decreased in rheumatoid arthritis. 4) The serum proteins of 17 patients with rheumatoid arthritis underwent SH-SS interchange more readily when heated than the serum proteins of 32 patients with other diseases ($p < 0.01$). These biochemical abnormalities suggest that increased SH and SS group reactivity may play a part in the pathophysiology of rheumatoid arthritis.

Absorption and Metabolism of β -Carotene and Vitamin A

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Radioactive β -carotene and vitamin A were fed to rats with cannulated thoracic ducts, and also to patients in whom polyethylene cannulae had been implanted in the thoracic duct in the neck. Lymph was collected and the lipid extracted and analyzed by chromatography in several different systems. During absorption, the vitamin A was almost completely esterified, and the esters were absorbed via the lymph mainly incorporated in lymph chylomicrons. During absorption of β -carotene the carotene was largely converted to vitamin A, which was then esterified and transported in the lymph in a manner similar to that of dietary preformed vitamin A. After feeding labeled β -carotene, unchanged carotene comprised one fourth the absorbed radioactivity in human lymph, but could not be demonstrated in rat lymph. The fatty acid composition of the vitamin A esters was remarkably constant, regardless of the composition of the diet and regardless of whether the esters

were derived from dietary vitamin A or β -carotene. Vitamin A palmitate predominated in all samples, and saturated esters (vitamin A palmitate + stearate in a ratio of 2 to 1) consistently comprised about three fourths of the labeled esters.

The active biosynthesis of vitamin A from β -carotene can be brought about with cell-free homogenate fractions of rat intestinal mucosa. The reaction requires molecular oxygen and bile salts, and is stimulated by glutathione. The product, obtained in yields of up to 50%, has been identified as vitamin A aldehyde (retinal) via its semicarbazone derivative and also by gas-liquid radiochromatography. The reaction mechanism involves the central cleavage of β -carotene into two molecules of retinal. During this reaction the hydrogen atoms attached to the central carbons of β -carotene are completely retained. The conversion of β -carotene into retinal is most likely a dioxygenase reaction.

Human Leukocyte Phospholipids: Relation to Cell Type

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The lipid composition of human erythrocytes has been described in detail by a number of investigators, but little information has been available concerning the composition of human leukocyte lipids. In the present study, phospholipids of normal and leukemic leukocytes were compared with those of erythrocytes and platelets, by means of quantitative thin-layer chromatography on silica gel H. A distinct and characteristic lipid pattern was found for each of the cell classes of human blood. Moreover, comparison of total lipid weight, lipid phosphorus, phospholipid distribution of normal leukocytes (mixed population), and leukemic leukocytes of several types showed substantial differences.

Normal leukocyte suspensions were separated into lymphocyte and granulocyte fractions by passage through columns of small glass beads. The phospholipid composition of the isolated lymphocytes and granulocytes was then determined by quantitative thin-layer chromatography. These studies revealed considerable differences in the lipid pattern of lymphocytes and granulocytes, but no significant difference between normal and abnormal cells of the same morphologic class. Apparent differences in phospholipid patterns of normal and leukemic leukocyte preparations appear to be related primarily to the preponderance of a particular cell type.

Mechanisms of Inflammation, III: Laser-Induced Thrombosis Observations on the Nature of White-Cell Sticking and a Preliminary Report on the Effect of Heparin on Venous Thrombosis

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Heat-induced microburns, inflicted by a laser beam on a small vascular bed of a rabbit ear chamber, yield a highly controllable system for studying the formation, propagation, and dissolution of thrombi in the walls of venules. Under these circumstances the thrombus takes the form of a core of red cells, injured in the burst of laser energy, surrounded by a growing corona of white cells and platelets that adhere to the thrombus. The growth of the thrombus eventually results in occlusion of vessels leading to stasis and ultimate destruction of the vessel wall if the stimulus is severe. By reducing the intensity of the laser beam, lesions can be produced that last for a few hours only and then the same part of the same vessel can be restimulated under varying experimental conditions. Morphological evidence suggests that the lesion is an intravascular one, with the injury confined to the luminal side of the vessel wall, and there is little evidence

either of emigration of white blood cells or of vascular leaks at the site of injury. This suggests that these latter phenomena, characteristic of so many other types of experimental injury, may be mediated by extravascular factors, a point not heretofore appreciated due to the diffuseness of many forms of experimental injury, but possibly made evident by the discreteness of the laser injury. Because the system permits reproducible injuries in the form of thrombotic sticking at the same site in the same vessel, each thrombus clearing within a few hours, it is possible to test the effect of heparin on the prevention and modification of venous thrombosis. In a series of experiments involving several vessels in one ear chamber, under normal conditions and under heparinization as high as 10 mg./kg., no clear-cut distinction could be found in the character of thrombosis with or without heparin.

The Regulation of Globin Synthesis by Hemin

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The observation that hemin stimulates the synthesis of globin by reticulocytes obtained from iron-deficient rabbits was reported by Bruns and London (*Biophys. Biochem. Res. Com.* 18: 236, 1965). We have conducted further studies on the mechanism of this effect, using the technique of sucrose density centrifugation to isolate the ribosomal structures engaged in protein synthesis. By this means it is possible to demonstrate an increase in both the propor-

tion of polyribosomes engaged in protein synthesis as well as an increase in the specific activity of the polypeptide chains attached to them when iron deficient reticulocytes are incubated with 1×10^{-4} M hemin. Studies carried out in the presence of inhibitors of protein synthesis such as puromycin, cycloheximide, and *o*-fluorophenylalanine indicate that polypeptide chain formation is probably required in order for hemin to exert its effects.

The Effect of Ethacrynic Acid on Sodium Transport in the Toad Bladder

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The administration of ethacrynic acid (EA) to man results in a profound saluresis and diuresis. The drug is believed to inhibit sulfhydryl-dependent enzyme systems in the renal tubular cell. We have studied the effect of EA on the isolated toad bladder to determine whether its action could be reproduced in an *in vitro* system. The addition of as little as 10 μ g./ml. of EA to the serosal bathing medium resulted in a progressive fall in short-circuit current (an index of active sodium transport); 50 μ g./ml. decreased short-circuit current to 25% of its control value after one hour. There was no concomitant decrease in the permeability of the bladder to chloride-36. The addition of cysteine to the

medium stopped the progressive fall in short-circuit current produced by EA, presumably by competing with tissue sulfhydryl groups for the methylene group of the drug. In addition to its effect on sodium transport, EA decreased the vasopressin-induced net water movement across the toad bladder; this effect, however, was only seen after prolonged exposure to EA. Our studies indicate that ethacrynic acid is a potent inhibitor of sodium transport in the toad bladder, and support the view that EA is capable of reacting with sulfhydryl groups in renal tubular cells. Current studies are directed toward a further clarification of the mechanism of action of the drug.

Phospholipid Synthesis by Rabbit-Lung Tissue

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Earlier observations reported here have demonstrated that rabbit lung tissue can rapidly incorporate acetate into phospholipids. Subsequent experiments have revealed that this synthetic pathway can be demonstrated both *in vitro* and *in vivo*. It was also found that the preferential incorporation into phospholipids differs from other tissues, such as liver. It was furthermore observed that precursors such as glucose, lactate, pyruvate, and palmitate, are—at slower rates—also preferentially in-

corporated into phospholipids, specifically lecithin. The site of these reactions apparently resides in the large (special) alveolar cells as documented by autoradiography. The rapid and preferential incorporation of acetate into palmitate and lecithin may well be related to the presence in lung of di-palmityl lecithin. This unusual phosphatide has been identified as the predominant surface tension-lowering component of the lipoprotein layer which lines the air-tissue interface of mammalian alveoli.

The Relationship Between Metabolic Activity and the Capacity to Reduce Methemoglobin in Human Erythrocytes

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The ability of intact human RBC to reduce methemoglobin (methgb) to hemoglobin (hgb) has been shown to depend upon the metabolic activity of the cells and has been attributed, in part, to the direct nonenzymatic reduction of methgb by reduced glutathione (GSH). This relationship has been reinvestigated in normal human RBC treated with sufficient NaNO_2 to oxidize most of the hgb to methgb. The GSH concentrations in these nitrite-treated RBC were decreased by less than 10%. Aliquots of methgb-containing RBC were preincubated at 37° C. for one-half hour with 9 to 11 μmoles of N-ethyl-maleimide (NEM)/ml. of RBC to bind all of the residual GSH. The binding of GSH by NEM has been presumed to be irreversible. Subsequent incubation of the NEM-treated RBC with 10 $\mu\text{moles/ml.}$ of glucose or inosine for 5½ hours at 37° C. did not result in significant regeneration of GSH. Reduction of methgb in NEM-treated RBC with glucose as substrate, however, was markedly impaired, glucose utilization was decreased by about 50% and formation of

lactate plus pyruvate was reduced by about 75%. With inosine as substrate, reduction of methgb was inhibited by less than 10%, disappearance of the ribose moiety of inosine was not decreased, and the production of lactate plus pyruvate was diminished by less than 30%. The inhibition of methgb reduction by NEM when glucose was the substrate could be explained by decreased glycolysis, probably secondary to a direct effect of NEM on the activity of such essential enzymes as hexokinase and glyceraldehyde-3- PO_4 dehydrogenase. Utilization of inosine, which does not require hexokinase activity, would be impaired by NEM to a lesser degree and would permit nearly normal reduction of methgb. These results may be interpreted as indicating that GSH was not required directly for the reduction of methgb under the conditions of these experiments and that impairment of the capacity to reduce methgb to hgb in the presence of a low concentration of GSH resulted only from decreased glycolytic activity in the NEM-treated RBC.

The Immunologic Activity of the Nitro-Olefins

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Evidence has been presented to show that automobile exhaust contains conjugated nitro-olefins, a series of unsaturated hydrocarbons with a nitro group substituted on one of the doubly bonded carbons. The present studies deal with the immunologic activity of these hydrocarbons.

Guinea pigs responded by topical application or injection of 20 to 40 μ moles of representative nitro-olefins, 2-nitro-2-butene, 3-nitro-3-hexene, 4-nitro-4-nonene or 2-nitro-2-nonene, by manifesting a delayed hypersensitive reaction when these materials were reapplied 7 to 14 days later. The unsubstituted olefins, nonene and hexene, a nitro-paraffin, 3-nitro hexane, an unconjugated nitro-olefin 6 nitro-1-hexene and the paraffins hexane and nonane were also tested for immunologic activity. These compounds

failed to sensitize guinea pigs for later skin tests, and failed to produce reactions in animals sensitive to the nitro-olefins.

Immunologic cross-reactivity was observed between 2-nitro-2-butene and 3-nitro-3-hexene and also between 3-nitro-3-hexene and 4-nitro-4-nonene, but no cross-reactivity was found between 2-nitro-2-butene and 4-nitro-4-nonene. This evidence indicates that molecular size is important in the specificity of the delayed reaction.

Demonstration by paper chromatography of reaction products formed by the addition of nitro-olefins to cysteine solutions shows that these compounds are capable of covalent reactions with protein constituents. All previously described low molecular weight sensitizers have exhibited this property.

Blood Groups on Human Cells Growing in Culture

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Knowledge of the extent to which stability of blood groups on growing cells in culture can be controlled is of importance in assessing their role in somatic cell genetics. Observations of different blood groups known to be present on erythrocytes were carried out in primary amnion cells, in transformed amnion cells, and in five different strains of HeLa cells. Blood group activity, as determined by mixed agglutination of immunofluorescence was correlated with: 1) composition of nutrient medium, 2) cell morphology and function using malignancy or cell transformation as a criterion. Although differences of survival between various blood groups were noted in primary cells in culture, a general deterioration in activity was noted in conjunction with other manifestations of dedifferentiation. Augmentation of group B and H reactivity occurred in certain lines of transformed amnion cells and in group H reac-

tivity in HeLa cells following addition to customary media containing serum from nonhuman sources of chemical components known to be present in soluble blood groups. When other combinations of nutrient media were tested for effects upon blood groups, the presence of human serum exerted a specific inhibitory effect upon group B in a cell line that possessed this factor prior to the addition of human serum.

In the FD amnion line and in two strains of HeLa cells, which demonstrated individual cell variation in regard to group B, H, and Tja the heritable nature of these characteristics was suggested by corresponding antigen variations in cultured cell clones using the appropriate fluorescent labeled antisera as indicators. (*This investigation was supported in part by Public Health Service Research Training Grant TL-AI-247-03.*)

Pulmonary Transcapillary Water Exchange in the Intact Dog

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The distribution of water between the vascular and the interstitial compartments is governed by the Starling law of transcapillary exchange. A series of experiments was designed to investigate the relationship between water accumulation in the lung and the difference between hydrostatic and oncotic pressures in the pulmonary capillaries (PLA-COP). These experiments were also intended to show the role of nonmeasurable pericapillary forces in the formation of pulmonary edema. A pressure difference favoring the formation of edema was produced in 38 intact, spontaneously breathing dogs by inflating a balloon in the left atrium and then rapidly infusing saline to produce hemodilution. The rate of accumulation of lung water was measured during the experiment by an indicator-dilution technique, and by drying the lungs at autopsy. The rate of accumulation of water was determined in 8 dogs with low vascular pressure and low pressure difference for 2 hours (PLA 18 mm. Hg; PLA-COP 12 mm. Hg), in 12 dogs with high vascular pressure and high pressure difference for 1 hour (PLA 28 mm. Hg; PLA-COP 23 mm. Hg),

in 6 dogs with high vascular pressure and low pressure difference for 2 hours (PLA 29 mm. Hg; PLA-COP 13 mm. Hg), and in 12 dogs with surgical obstruction of right lymphatic duct drainage. The respective rates of water accumulation in these four groups were 1.0, 4.0, 1.6, and 1.8 ml./kg. b.w. \times hours \times 10 mm. Hg pressure difference. The only significant difference noted was that between the groups with the low and with the high transcapillary pressure difference (PLA-COP), indicating that the relationship between lung water accumulation and the gradient PLA-COP is non-linear. In two of the eight animals with low pressure and low gradient there was no net increase in lung water during the experiment. It is concluded that pulmonary transcapillary water exchange is influenced by pericapillary forces that tend to oppose filtration at low transcapillary gradients and to enhance filtration at high gradients, and that right lymphatic duct drainage and the absolute level of vascular hydrostatic pressure do not influence significantly the accumulation of lung water in acute experiments.

Pathogenesis of Candidiasis

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The kidney is the tissue of maximum involvement after intravenous infection with *Candida albicans* or *C. tropicalis*. Serial histologic studies indicate 2 major factors in renal susceptibility: 1) the ability of these species to rupture into and grow within the protected environment of the renal tubular lumen unhampered by host cellular defenses, and 2) a 4-hour delay in mobilization of polymorphonuclear leukocytes into the kidney. Additionally, *C. albicans* resists killing after phagocytosis; this species forms hyphae within the leukocyte, and then grows out of the leukocyte, presumably facilitating entrance into the renal tubular lumen. During the past 4 years we have studied a substance in plasma that is lethal for *C. albicans*. The sera from about 600 persons have been analyzed. The results

show that patients with juvenile diabetes, hepatic disease, azotemic renal disease, and leukemia are deficient in this substance, as are the majority of those with either mucocutaneous or systemic candidiasis. The substance appears to be a small protein or polypeptide migrating with the alpha and beta globulins. In most patients with inactive sera, small amounts of inactive sera reduce profoundly the killing capacity of normal sera. Furthermore, dilution of inactive sera 1:5-1:10 frequently restores activity. Susceptibility or resistance to *Candida* invasion may well be related to the activity or inactivity of this plasma substance. The data further suggest that for the most part inactive sera do not lack candidacidal activity but rather have some interfering substance.

Regulation of Venous Return in a Hepatic Valve: Two-Chamber Model

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Let G be a time-varying parameter determined by a respiration-regulated hepatic valve in a two-chamber model of venous return where arterial inflow pressure almost dissipates through isolating resistances and common outflow pressure varies 180° out of phase with the operation of the valve. Let the two chambers be the splanchnic and systematic segments of venous return. Then, whenever G varies, splanchnic and systemic returns grow in opposite directions and

there is an alternating transformation of kinetic and potential energy. Now, for an observer at the inflow of the chambers beyond the arteriolar resistance, this mode of operation will imply less impedance to total venous return than would obtain from a more conventional, synchronous two-stroke mode in a valveless model. Physically, there is more effective use of energy liberated during respiration.

The Mode of Timing of DNA Synthesis and Mitosis in Mammalian Cells

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It has been postulated repeatedly, with little support from experimental evidence, that the deoxyribonucleic acid (DNA) synthesis period (S-period), the G2-period, and the period of observable mitosis (M-period) have each fixed durations, and that differences in generation time are due to expansion or contraction of the G1-period alone. Evidence in favor of an alternative mode of timing of DNA synthesis and mitosis is presented. This new evidence comes from asynchronous mouse ascites mast cell (strain P815Y) and L-929 mouse cell cultures grown at many different rates in chemostat and cytogenerator. An indirect method eliminating undesirable thymidine- H^3 effects was used for determination of the chronology of the cell-division cycle. The experimental data indicate clearly that

S-period, G2-period, M-period and, consequently, also G1-period each expand homogeneous-linearly with increasing generation time ("proportional timing mode"). Estimation of the invariant fractions of generation time spent by P815Y cells in G1-, S-, G2-, and M-period, as 0, 0.8184, 0.1425 and 0.0391 respectively, shows total absence, under conditions of exponential growth, of a detectable G1-period in the cell-division cycle of a highly virulent neoplastic cell. "Proportional timing" of the four discrete phases of the mitotic cell cycle, it is shown, preserves, during exponential cell duplication, the characteristic temporal order of specific macromolecular syntheses at all possible duplication rates. (*Supported by Contract Nonr-266(76) from the Office of Naval Research.*)

Infrared Spectroscopic Examination of Aorta and Other Tissues by the Attenuated Total Reflectance Technique (ATR)

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Various solid sampling techniques are usually used with transmission spectroscopy for the determination of infrared spectra of biological tissues (*Anal. Biochem.* 9: 1, 1964; and H. Szymanski, ed., *Progress in IR Spectroscopy*. New York, Plenum, 1964, vol. 2, p. 213). Less contaminating, more rapid, and more convenient methods are now available. Solutions of peptides have been examined by the attenuated total reflectance (ATR) technique (*Nature* 200: 1093, 1963); and beef tendon has been studied by the frustrated multiple internal reflection (FM-IR) method (*Appl. Spectroscopy* 18: 7, 1964). Also, FMIR has been used to study rat tissues (*Anal. Biochem.* 12: 406, 1965). The present report compares the ATR spectra of autopsy specimens of human neonatal aorta and adult atheromatous aorta. A thallium bromide-iodide hemi-

cylinder was used in the ATR unit on a Perkin-Elmer model 521 infrared spectrophotometer. A wire screen was used in the reference beam. Some absorption bands are common to both types of tissues, e.g., 3300 cm^{-1} NH stretch, 1540 cm^{-1} NH bend (present in protein as CONH amide II), 1460 cm^{-1} CH band in CH_2 and CH_3 (protein and lipid). However, the adult atheromatous aorta shows absorption bands that are either not present or are much less intense in the neonatal aorta, e.g., 1235 cm^{-1} P = O stretch (probably phosphate component of phospholipid, RNA, or DNA), 2845 and 2918 cm^{-1} CH stretch (possibly cholesterol and esters), 1745 cm^{-1} carbonyl stretch and 1160 cm^{-1} COC stretch, presumably from cholesteryl esters and phospholipids. Human liver, spleen, lung, endometrium, and rat heart were also examined.

Relationship of Alcohol Consumption to Vitamin Deficiency in the Rat

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The consumption of alcohol produces symptoms that bear a relationship to the nutrition and vitamin status of animals. Alcohol is tolerated better by animals in good nutritional status. In a preliminary experiment the levels of tolerance for alcohol ingestion were determined. Rats were subjected to various percentages of alcohol as drinking water. Other animals were given alcohol with 2% sucrose added to make the mixture more tolerable. It was found that animals would tolerate as high as 20% alcohol, and this was chosen as the percentage to be used in future experiments. The addition of sucrose to the solution was not deemed necessary and, indeed, proved to be a complicating factor. In the following experiment the relationship between vitamin deficiency and alcohol consumption was investigated. Rats placed on a vitamin A-deficient diet were given 20% alcohol as drinking water and the progress of the deficiency compared with rats on de-

ficient diet but given pure water. Weanling rats placed immediately on a vitamin A-deficient diet showed gross signs of the deficiency in 30 to 40 days if given pure water. Animals on 20% alcohol and a deficient diet always show signs of the deficiency earlier, gain less weight throughout the experiment, and succumb to vitamin A deficiency 8 to 12 days earlier. Thus alcohol accelerates the progress and severity of the vitamin A deficiency. Although chronic alcoholic states produce certain liver symptoms, these symptoms do not appear in the short-term alcohol-deficiency studies and therefore cannot be implicated in the earlier death of such animals. The comparison of liver stores of vitamin A and serum blood levels during the progress of the deficiency suggests that alcohol consumption causes a release of vitamin A from the liver to the blood, which seems to be the most important factor in producing early and more severe symptoms of the deficiency in animals.

Histocompatibility Studies in Man

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Cytoplasmic fractions of blood leukocytes act as potent transplantation antigens in man. The response to such preparations is dependent upon the dose of antigen used. It may also be conditioned by histocompatibility determinants detectable by leukocyte grouping techniques.

Small doses of transplantation antigens induced accelerated or white graft rejection of skin allografts obtained from the same donor. A sixfold increase in the antigen dose resulted in relative prolongations of survival of such allografts. Transplantation antigens pooled from 40 different individuals cross-sensitized the recipients to skin allografts obtained from other unrelated donors.

The possibility that such cross-reactions were an expression of the existence of transplantation antigen groups in unrelated human subjects was tested by correlation of leukocyte surface antigen groups *4a4a*,

4b4b, and *Mac* with the response of specifically group-selected individuals to skin allografts. Sensitization with leukocytes or skin grafts obtained from leukocyte group-incompatible donors induced in the recipients an accelerated or white graft rejection of skin allografts obtained from other donors selected on the basis of the same leukocyte group incompatibility. Skin allografts obtained from leukocyte group-compatible donors were accorded first-set survival times in such recipients.

Results of this study indicate that leukocyte group antigens may play an important role in conditioning histocompatibility responses in man. They suggest an approach to the selection of donor-recipient combinations for further studies of the facilitation of skin allograft survival in man by pretreatment with suitable preparations of transplantation antigens.

Single Unit Activity in Human Epileptiform Spike Foci: Results with Two Independent Microelectrodes

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Single-unit and slow-wave activity in human epileptogenic cortex has been recorded to delineate the nature of the neuronal abnormality in the EEG spike focus, the relation of cortical unit discharge to the epileptiform surface spike, and the intracortical distribution of potential; also to provide a reference base for experimental models of focal epilepsy. Investigations with one microelectrode revealed the existence of two different types of epileptiform spike, demonstrated no intrinsic neuronal abnormality in cortical spike foci, and suggested that EEG spikes may depend on the activation of cortical mechanisms related to evoked potentials (*Bull. N.Y. Acad. Med.* 41: 239, 1965). This evidence being consistent with the possibility that clinical focal epilepsy comprises a functional aberration of neuronal organizations, simultaneous recording with two adjacent intracortical microelectrodes has been carried out to observe the activity of distinct neurones or

neuronal clusters in epileptogenic cortex.

A double micromanipulator provided independent penetration of two micropipettes in the same vertical plane. Sites of microelectrode penetration were selected from critical electrocorticographic mapping of greatest epileptiform spike activity in craniotomies for the treatment of drug-refractory seizures. Synchronization of unit activity was demonstrated during a brief paroxysm from activation with intravenous pentylenetetrazol. Spontaneously recurring epileptiform spikes were associated with synchronization of unit activity, although the mechanism of this synchronization was separable from that which generated the EEG spike. Synchronization of unit activity was observed also in the absence of epileptiform waves.

These findings modify current concepts of human focal epilepsy, suggesting a formulation that requires only a local defect of (inhibitory?) synaptic function.

A Comparison of the Lipid Composition of the Gangliosides of Maturing Normal and Tay-Sachs Diseased Brain

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A study was made of the nature of the sphingosine and fatty-acid portions of the gangliosides of maturing normal brain with a view to furthering an understanding of the role of these water-soluble glycosphingolipids of the neural cell and the reasons underlying their massive accumulation in the degenerating brain in familial amaurotic idiocy (Tay-Sachs disease). The fatty acids were removed from gangliosides by transmethylation and their composition studied by gas-liquid chromatography. The structure of the sphingosine portion was determined by oxidative degradation. Rat brain gangliosides had 18-carbon sphingosine at birth. With brain maturation, the gangliosides accumulated 20-carbon sphingosine until there were almost equal amounts of each base. Rat-brain cerebro-

sphingomyelin had only 18-carbon sphingosine at all stages of development. Fatty acid components of the sphingolipids changed with brain maturation. Stearic acid in the gangliosides rose initially, then continually declined. An over-all decline in stearic acid was also found in the cerebro-sides and sphingomyelin of rat brain. Results with human brain gangliosides were similar to those with rat-brain gangliosides. Tay-Sachs gangliosides had a very high stearic acid content and almost no 20-carbon sphingosine. Thus they resembled the gangliosides of normal fetal brain rather than those of the age group from which the diseased brain specimens were obtained (2 to 3 years of age). The accumulation of fetal-type gangliosides may be related to the pathogenesis of Tay-Sachs disease.

A Common Antigenic Determinant in Two Different Human Proteinpolysaccharides

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Anionic polysaccharides such as chondroitin sulfate and hyaluronate are important constituents of the ground substance of connective tissue. Protein-free preparations of chondroitin sulfate and hyaluronate are not antigenic. It has now been established that chondroitin sulfate in human cartilage and hyaluronate in human synovial fluid are firmly bound to noncollagenous proteins forming compounds designated protein-polysaccharides (PP). Rabbits were immunized with either human cartilage PP or the PP of normal human synovial fluid, hyaluronateprotein (HP). Antibodies to the protein moiety of cartilage PP were demonstrated by three different immunological methods, i.e., precipitation, agglutination, and immunofluorescent staining of cartilage. Antibodies to the protein moiety of HP were demonstrated by electrophoretic immobilization and immunofluorescent staining of human synovial membrane. An un-

expected finding was an immunologic cross-reaction between the protein moieties of PP and HP. Antibody to cartilage PP formed precipitin lines with HP and caused fluorescent staining of the lining cells of synovial membrane. Antibody to HP agglutinated sheep RBCs coated with cartilage PP and caused fluorescent staining of the chondrocytes in cartilage. Although PP and HP cross-react, they are not identical immunologically. Antiserum to HP absorbed with cartilage PP still produced fluorescent staining of the lining cells of synovial membrane; and antiserum to cartilage PP absorbed with HP still agglutinated sheep RBCs coated with cartilage PP. A cross-reacting antigenic determinant in the protein moieties of two different connective tissue proteinpolysaccharides is a new finding with exciting implications regarding possible structural similarities of these two compounds.

Stimulation of Anaerobic Function in Mammalian Tissues

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Although oxidative metabolism is felt to be essential for normal liver cell function, it was felt that anaerobic ATP production could be stimulated by metabolites capable of acting as electron acceptors. $\text{Na}^+\text{-K}^+$ transport was selected as a model energy-requiring system to be used as a physiologic assay.

Following a period of precooling, it was found that liver slices could accumulate K^+ and extrude Na^+ at 37°C . under a nitrogen atmosphere when either oxalacetate or pyruvate was added to the incubation mixture. The extent of active transport observed was comparable to that obtained under aerobic conditions. The stimulation afforded by oxalacetate and pyruvate was

abolished by ouabain.

Experiments using the metabolic inhibitors iodoacetate and dinitrophenol, as well as those employing 24-hour-starved rats, suggest that the increase in anaerobic cell function observed is the result of a stimulation of glycolysis.

Oxalacetate was also found to be effective in enhancing the ability of isolated perfused rat hearts to beat under anaerobic conditions.

It appears that there is a latent capacity for anaerobic energy production in the tissues studied which, when properly stimulated, can result in appreciable endergonic function in the absence of oxygen.

Effects of Milk and Cream Meals on Luminal Acidity of the Duodenal Bulb in Dogs

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The purpose of this study was to characterize the effects of hourly milk and cream meals on luminal acidity of the first part of the duodenum. The subjects were healthy dogs with leakproof prosthetic fistulas that provided access to the duodenal lumen, 3 cm. from the pylorus. Continuous recordings of pH of the duodenal bulb contents *in situ* (pH_{dbc}) were made after insertion of a combination (measuring and reference) glass electrode—enclosed in a fenestrated Teflon guard—through the fistula, and confirmation of the electrode position in the bulb by a novel endoscopic technique. Degree of luminal acidification was quantified in terms of aggregate duration of pH_{dbc} excursions below 3.0 and 4.0 in each half-hour interval and by planimetric measurement of the acidic excursions. In Group I experiments, after 1 hour of recording under “basal” fasting conditions, hourly feed-

ings of 100 ml. of whole milk and of “half-and-half” (milk and cream), alternately, produced substantial increases in duodenal-bulb acidity (over basal fasting control levels) in both the first and second half-hour intervals after each feeding. In Group II a state of moderate gastric hypersecretion was maintained throughout each experiment by constant intravenous infusion of $0.1\text{ }\mu\text{g}$. of histamine/kg. body weight/min. Here, compared with the high-fasting control level, the degree of duodenal acidification was decreased in the first half-hour after each feeding. But in the second half-hour it was usually considerably *greater* than the control level. The findings suggest the possibility that this traditional dietary regimen may be detrimental in treatment of duodenal ulcer if it is not adequately supplemented by antacid intake at the proper time between feedings.

Metabolic Cost of Minor Changes in Heat Balance of Small Neonates

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The subjects of this study were small, asymptomatic, newborn infants. Each infant was exposed to three experimental conditions in which the abdominal skin temperature was regulated by a radiant heat system: 35°, 36°, and 37° C. After complete temperature equilibration expired air was collected in a recording spirometer. The concentrations of O₂ and CO₂ of inspired and expired air were analyzed in a gas chromatograph. Arterial blood was obtained for determination of acid-base status. Body temperatures (colonic and six skin sites) were recorded, and average body temperature was computed using the formula: $0.4 (0.23 T_{\text{head}} + 0.33 T_{\text{abdomen}} + 0.11 T_{\text{arms}} + 0.23 T_{\text{thighs}} + 0.05 T_{\text{hands}} + 0.05 T_{\text{feet}}) + 0.6 T_{\text{colon}} = T_{\text{B}}$. Mean O₂ consumption was lowest (7.9 ml. *per infant* per minute \pm 0.25 S.E.) when the skin temperature was controlled at 36° C. (T_{B} 35.8° \pm 0.38 S.E.) As compared with this thermal condition, the most frequent response to equi-

libration of body temperature at the lower and higher levels was a small increase in metabolic rate. In the 35° C. condition (T_{B} 34.8° C \pm 0.3 S.E.) the mean increase (10.9% for 1° C. reduction T_{B} \pm 4.08% S.E.) was greater than would reasonably be expected to occur by chance. The mean increase in O₂ consumption that occurred when the abdominal skin temperature was controlled at 37° C. (T_{B} 36.8° C. \pm 0.3 S.E.) was smaller (5.9% for 1° C. increase T_{B} \pm 3.5% S.E.), and this rise in O₂ consumption might more easily be explained by chance. These results suggest that a neutral thermal state in very small neonates can be achieved when ambient conditions are adjusted to maintain abdominal skin temperature close to 36° C. The present studies support the results of clinical trials in Baltimore and Pittsburgh that demonstrated improved survival in the first days of life among small infants whose abdominal wall temperatures were controlled at 36.1° C.

Further Studies on the Mode of Action of Levorphanol

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We have previously found that the narcotic levorphanol inhibits selectively the synthesis of ribosomal RNA in *E. coli*. Recent studies indicate that the effectiveness of levorphanol increases as the Mg⁺⁺ concentration of the medium is lowered. However, evidence has been accumulated demonstrating that the inhibitory effect of the drug is not mediated via chelation of Mg⁺⁺. Inhibition is not abolished at Mg⁺⁺ concentrations 6 to 10 times higher than the levorphanol level. Levorphanol was shown to be a poor chelator by its inability to dissolve Mg₃(PO₄)₂, to remove Mg⁺⁺ from its complex with Eriochrome Black T, or to lower the pH of a neutral solution of MgCl₂. Work has also been carried out testing the possibility that levorphanol exerts its effect by preventing the reaction of unstable ribo-

somal precursors with ribosomal protein. The "relaxed" *E. coli* mutant 58-161 was starved of methionine in the presence of C¹⁴-uracil to cause the formation of labeled 18-25S "relaxed" particles. It was shown by sucrose gradient centrifugation that levorphanol did not prevent the conversion of "relaxed" particles to complete ribosomes upon the addition of methionine. The action of levorphanol has been compared with that of low levels of actinomycin D in *Bacillus subtilis* and *E. coli*. Actinomycin was found to inhibit the average rate of synthesis of messenger RNA and ribosomal RNA equally. It is concluded that the two drugs act by different mechanisms. (*Supported by grants from the Public Health Service and The Health Research Council of the City of New York.*)

Compensatory Growth in Tissue Culture

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Explants of fetal mouse skin in plasma clot were grown in normal media, and then in media modified by the addition of cortisol or the removal of bicarbonate in an attempt to inhibit growth. A third period in normal media followed for observation of possible acceleration after inhibition. The surface area of each explant was measured daily. The data obtained were examined by analysis of covariance and from the slopes

of regression curves comparing treated and control explants. Both methods of analysis revealed inhibition of growth ($p = < 0.05$) from deprivation of bicarbonate or treatment with 100 to 200 $\mu\text{g./ml.}$ cortisol for 2 days. Compensatory growth after deprivation of bicarbonate did not occur, but acceleration was demonstrated in explants treated with 50 $\mu\text{g./ml.}$ cortisol, an amount too small to induce detectable inhibition.

Remission in Cushing's Syndrome with o,p'-DDD

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o,p'-DDD was administered to two patients with Cushing's syndrome (one with nontumorous adrenocortical hyperfunction and one with metastatic adrenal carcinoma). The patients were treated for 199 and 125 days respectively. In the subject with nontumorous adrenocortical hyperfunction the studies were continued for 143 days after discontinuation of therapy. The administration of o,p'-DDD was associated with a striking clinical and biochemical remission. There was a prompt reduction in the urinary excretion of 17-hydroxycorticoids (17-OH-CS), and in the early weeks of therapy this occurred in the absence of a commensurate decrease in the plasma levels of 17-OH-CS and in the cortisol secretion rate. The diminution in urinary 17-OH-CS correlated well with a decrease in the percentage of injected cortisol excreted as the glucuronide and in the urinary excretion of the tetrahydrocorticoids. The reduction in the tetrahydrocorticoids was

associated with an almost quantitative increase in the "free" or unconjugated steroid fraction. The latter fraction consisted of 6 β -hydroxycortisol and other highly polar Porter-Silber chromogens. More prolonged treatment with o,p'-DDD resulted in a marked fall in the cortisol secretion rate. These data indicated that o,p'-DDD affected both the adrenal production and the extra-adrenal metabolism of cortisol. During the early treatment period the latter effect predominated. A clinical response was observed in both subjects before a significant decrease occurred in the adrenal secretory rate of cortisol. The mechanism of the response during the early treatment period was not clear, although it appeared to be temporally related to the extra-adrenal effect of the drug. (*This investigation was supported by Public Health Service Research Grant AM-06431 from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.*)

Cellular and Subcellular Components Involved in Immunological Memory and Antibody Formation

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Recent experiments have demonstrated that antibody protein is formed on ribosomes from a pool of free amino acids, according to mRNA coded from chromosomal DNA. In view of these considerations, attempts were made to modify the degree of immunization by altering the quantity of nucleic acid present in the cells at the time of primary and secondary exposure to antigen in BDF1 mice.

High doses of actinomycin D produced a state of immunological unresponsiveness that continued for as long as the treatment was maintained. As many as 20 injections of antigen were given without inducing primary immunization. As soon as the actinomycin D treatment was discontinued, primary immunization could be readily induced. On the other hand, actinomycin D

did not prevent rapid antibody production in immunized animals receiving challenging injections of antigen.

These experiments indicate a variation in the essential role of RNA in the two states of immunity. During primary immunization a reduction in RNA synthesis reduced or prevented the formation of immunological memory. During the anamnestic response a reduction in RNA synthesis had little or no inhibitory effect upon induced antibody formation. Additional data will be presented indicating that mRNA necessary for antibody formation is inactivated by antigen during primary immunization, stored in reticuloendothelial cells during the interim, and finally released and incorporated into plasma cells during the anamnestic response.

Renal and Respiratory Adjustments to Chronic Hypercapnia

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The evolution and resolution of hypercapnia in man are accompanied by adjustments in the responsiveness of ventilation and the urinary excretion of HCO_3^- and H^+ that may be affected by the electrolyte composition of blood and tissues. In this study, the renal and respiratory adjustments to the hypercapnia of intrinsic respiratory disease (pulmonary emphysema) and musculoskeletal diseases of the thorax (kyphoscoliosis and muscular dystrophy) were studied during: 1) chloride depletion (dietary restriction and mercurial diuresis), 2) chloride repletion (KCl 100 mEq. for 5 days), 3) H^+ loading (NH_4Cl 100 mEq. for 5 days) and, 4) hyperventilation (mechanical respirator 4 to 6 days). Electrolyte composition of urine and serum, H^+ excretion, arterial blood gases, and ventilatory responsiveness were measured during metabolic balance. Mild chloride depletion in chronic hypercapnia induces increases in serum bi-

carbonate without significant effects on blood gases or ventilatory responsiveness. Severe chloride depletion results in metabolic alkalosis, a reduced ventilatory responsiveness, and increased hypercapnia and hypoxemia. Cl^- repletion reverses these effects. NH_4Cl decreases serum HCO_3^- with no increase in urinary HCO_3^- . For similar changes in extracellular (H^+), NH_4Cl induced greater changes in ventilatory responsiveness than did KCl . This difference is attributed to the larger distribution of H^+ intracellularly by NH_4Cl . During chronic hyperventilation, extracellular bicarbonate losses may be accounted for by renal bicarbonate excretion rather than by intra- and extracellular HCO_3^- redistribution. These studies demonstrate that changes in (H^+) and (Cl^-) can induce significant adjustments in ventilatory responsiveness despite severe ventilatory restriction.

Clinical and Laboratory Evaluation of Patients with Hodgkin's Disease Complicated by Herpes Zoster Infection

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The depressed resistance of patients with Hodgkin's disease (HD) to the varicella virus has been known since 1924, when H. K. Pancoast and E. P. Pendergrass called attention to this association. Since 1951, 135 cases of HD have been seen at this hospital. Their average age was 43 years; the male:female ratio was 1.45; 120 showed HD granuloma; and the 102 with Stage III disease had a 50 per cent survival probability of 22 months.

Seventeen of these patients had 19 episodes of herpes zoster (HZ). Their average age was the same as for all HD patients. The male:female ratio was 0.89. In two, HZ preceded HD by 5 and 1 year, respectively. The other 15 patients had Stage III disease at time of HZ. A second episode of HZ occurred 18 and 10 months following the first attack in two women. Fifteen of 17 episodes of HZ occurred during active phases of HD. Three patients were in the first quarter of their HD, four in the second, two in the third, and eight in the final

quarter. Distribution of HZ was: cranial nerves (n.)—1, cervical n.—1, thoracic n.—2, lumbar-sacral n.—7, disseminated—5, and unknown—1. Therapy of HD immediately preceding HZ included: radiotherapy, 4 of 17; alkylating agents, 6 of 17; and none, 7 of 17 episodes. Glucocorticoids were administered prior to 3 of 11 local and 4 of 5 disseminated HZ attacks. Leukopenia ($< 4000/\text{c.mm.}$) accompanied by granulocytopenia ($< 3150/\text{c.mm.}$) occurred in 4 of 17 episodes. Lymphopenia ($< 1500/\text{c.mm.}$) was noted in 10 of 17 HZ infections. Hypogammaglobulinemia was found in 6 of 9 episodes of HZ in the seven cases in whom a serum protein electrophoretic pattern was obtained. In two patients, the HZ infection was complicated by a secondary bacterial infection. Four of five patients with disseminated HZ died of HD within 6 months (average 2.5 months) and 7 of 10 patients with localized HZ died of HD within 12 months (average 9.3 months) of the occurrence of this complication.

Production and Prevention of Hemorrhagic Atelectasis in Isolated Perfused Canine Lungs

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The cause of hemorrhagic atelectasis and bronchopneumonia following pump-oxygenator perfusion remains obscure. To study the pathogenesis of these lesions, isolated, ventilated canine lung pairs were perfused in circuit with heat and gas exchangers under varying conditions of blood age, temperature, and preservation. Pulmonary pressures, resistances, flows, and function were monitored before and during perfusions. Following perfusion, lungs were studied radiographically, angiographically, grossly, and microscopically.

Using 1-day-old citrated canine blood at 26°C. 10 lung pairs were perfused up to 6 hours with preservation of functional, hemodynamic, and anatomic integrity. In striking contrast, 10 perfusions of 1 to 2

hours at 37° with 21-day-old heparinized blood resulted in severe hemorrhagic atelectasis, markedly impaired gas exchange, and increased vascular resistance. Eighteen perfusions with fresh heparinized or old citrated blood at 26 degrees produced varying degrees of hemorrhagic atelectasis after 2 to 3 hours.

These results indicate that homologous blood age, temperature, and heparin anticoagulation may be additive in releasing substances that produce pulmonary damage or in destroying factors that protect lung tissue. Production and prevention of hemorrhagic atelectasis in isolated perfused lungs is an initial step in the identification of these substances or factors.

Assembly of Ribosomes in HeLa Cells

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Although the central role of the ribosome in the synthesis of proteins is widely recognized, little is known about the assembly of the ribosome itself from its constituent RNA and protein molecules. We have studied this process in HeLa cells by examining the appearance of the ribosomal structural protein on mature ribosomes purified from the cell's cytoplasm. Since the site of assembly of the ribosome is the nucleus, the completed product can be prepared free of any precursors. Radioactive ribosomal structural proteins, synthesized during a short pulse of radioactive amino acids, continue to emerge from the nucleus as part of newly completed ribosomes for

a period of 2 hours. To ascertain whether this was due to a complex assembly process or simply to pools of ribosomal proteins, the proteins of ribosomes isolated at various times after the pulse were examined by means of polyacrylamide gel electrophoresis. It was concluded that the ribosome is made up of large numbers of proteins, of which there are two classes: those that are in equilibrium with cytoplasmic proteins, and those that emerge from the nucleus associated with RNA. The latter are assembled onto the RNA in a time that is short compared to that necessary for an RNA molecule to develop into a mature ribosome.

Fidelity in Translation of the Genetic Code

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A major concept in biochemical genetics is that the sequence of bases in messenger RNA (mRNA) codes for the amino-acid sequence in a related protein. The term "fidelity" refers to the precision with which this translation occurs. In a subcellular system from *Bacillus stearothermophilus*, low temperature and several polycations cause miscoding in the translation of various synthetic mRNAs. Similar studies were performed with subcellular reticulocyte and rat-liver extracts. Though poly U codes for phenylalanine incorporation in these animal systems, efforts to enhance the ability of poly U to miscode for leucine or isoleucine were without effect. These efforts included varying the duration or temperature of incubation; changing the concentrations of poly U, magnesium, or potas-

sium; adding ammonium ion, streptomycin, dihydrostreptomycin, spermine, spermidine, or putrescine; and varying the amino-acid composition of the reaction system. The influence of these same variables on fidelity of hemoglobin synthesis was also studied in the reticulocyte system. Though each of the variables affected the over-all rate of the reaction, they did not cause a relative increase in the incorporation of leucine or phenylalanine. Therefore, translation of the genetic code proceeds with higher fidelity in mammalian cell extracts than in bacterial systems. The fidelity of protein synthesis in intact cells and the possibility that miscoding functions *in vivo* as a regulatory mechanism are being evaluated in current studies.

Studies on the Electroencephalographic, Auditory-Evoked Responses and Behavioral Correlates of Sleep of the Premature Infant

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Behavioral observations during two 3-hour periods between feedings of 25 premature infants at different gestational ages demonstrates that the "quiet" sleep phase progressively occupies a greater per cent of the total sleep time compared with the "active" stage as maturation occurs. Electroencephalographic (EEG) correlates of this maturational process reveals that after 30 to 33 weeks estimated gestational age (EGA) a differential pattern can be recognized between the two behavioral sleep phases, active and quiet. Prior to this age, the EEG is generally composed of high-voltage sharp wave bursts lasting 1 to 2 seconds, alternating with periods of very low voltage activity lasting up to 30 seconds. Algebraically-summed auditory-evoked responses from scalp recordings were found in all infants studied; the youngest EGA was 26 weeks. Analysis of latency and amplitude of wave

components of the evoked responses from multiple scalp areas revealed a series of complex changes as maturation proceeds. Younger infants had a predominantly large negative wave with a latency of 200 to 250 msec. with longer latencies from anterior than from posterior midline electrode placements. Earlier posterior components and more complex longer latency components emerged as maturation progressed. Clear differences in the pattern of the evoked response were noted for these maturational changes between anterior and posterior scalp regions. (*This investigation was supported by Public Health Service Research Grants NB-03356 and HD-01405 from the National Institute of Neurological Diseases and Blindness and the National Institute of Child Health and Human Development, respectively.*)

The Metabolism of ^{14}C -Labeled Disaccharides Following Intravenous Injection in the Rat

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Increased amounts of unhydrolyzed disaccharides are absorbed through the intestinal mucosa in several gastrointestinal diseases. For this reason the metabolic fate of circulating disaccharides was investigated in nonfasting rats. Following the intravenous administration of 5 mg. (0.5 μc .) of ^{14}C -labeled disaccharides, the radioactivity of expired CO_2 and urine was measured for 24 hours. Results are shown as means \pm S. D. in the accompanying table.

Sugar	$^{14}\text{CO}_2/24 \text{ hr.}$ % dose	Urine $^{14}\text{C}/24 \text{ hr.}$ % dose
Glucose-1 ^{14}C (5)	62.0 \pm 11.6	5.3 \pm 4.7
Glucose-U ^{14}C (5)	64.0 \pm 12.0	14.8 \pm 10.3
Maltose-1 ^{14}C (5)	54.6 \pm 7.0	4.8 \pm 3.9
Maltose-U ^{14}C (3)	61.6	6.0
Lactose-1 ^{14}C (6)	6.2 \pm 2.7	62.1 \pm 13.5
Sucrose-U ^{14}C (5)	7.6 \pm 2.4	68.4 \pm 10.8

Lactose and sucrose were poorly oxidized to CO_2 and were largely excreted unchanged in the urine. Maltose, however, compared favorably with glucose as a circulating substrate for oxidation to CO_2 . This was true even in animals who had resection of their small intestine. For this reason, maltase activity was measured in homogenates of other rat organs and compared with intestinal mucosa. Relative activities (units)* were: intestinal mucosa-495, kidney-17, brain-4, liver-2, spleen-1, skeletal muscle-0.1, and serum-91. Hepatectomy and bilateral nephrectomy did not affect the oxidation of circulating maltose to CO_2 in the intact animal.

It is concluded that circulating maltose, but not lactose or sucrose, is significantly oxidized to CO_2 in the rat and that circulating maltose may be hydrolyzed by serum maltase.

*One unit equals 1 μmole maltose hydrolyzed/min./g. tissue protein.